PROLINE CONTENT OF UNITED STATES HONEYS

JONATHAN W. WHITE, JR., AND OREST N. RUDYJ*
*Eastern Regional Research Center**, Philadelphia, PA 19118, USA*

Summary

The proline content of 740 U.S. honey samples in two groups was determined. For 482 samples from 1974-75, the mean was 48·3 mg/100 g (S = 18·6, range 14·8-139). A group of 258 samples from 1956-57 averaged 54·1 mg/100 g (S = 21·9, range 16·9-148). The average loss from 4 months storage at 37°C of 18·6% is relatively minor in comparison with the normal variation found among honey samples. A significant inverse relationship was observed between the solids content of syrups as fed to confined bees and the proline content of the material stored by the bees.

Introduction

Although some attention had been given previously to the free amino acids of honey, little was known of their identity until paper chromatography was used. Komamine (1960) first noted that proline predominated in the two samples he analysed. Maeda et al (1962) confirmed this using ion-exchange chromatography, finding 50-66% of the total free amino acids to be proline. In the past eleven years, eight studies of the amino acids in honey by ion-exchange chromatography have been published, and all agree that proline predominates.

Table 1 summarizes the values from several of these investigations. Although Komamine suggested that pollen, with its high proline content, is the source of the free amino acids, later investigators generally agree that they originate in the honeybee (*Apis mellifera*). Proline predominates in stores from the sugar-feeding of bees, as it does in honey. Bergner and Hahn (1972) relate the proline content to the extent of manipulation by the bees in converting nectar into honey, which depends upon nectar water content and environmental conditions.

We have analysed two extensive groups of honey samples for proline content, to enlarge the data base on the composition of honey which can be used in distinguishing genuine from adulterated honey.

TABLE 1. Proline content of honey (mg/100 g)

No. samples	*Mean*	*Range*	*SD*	*Reference*
8		24·6-123·2		Curti & Riganti, 1966
9	14·6	12·5- 17·1		Michelotti & Margheri, 1969
26	42·6	16·1- 89·7	19·9	Bergner & Hahn, 1972
9	46·9	22·6- 81·7	21·2	Petrov, 1974
98	59·6	15·5-125·7	26·8	Davies, 1975*
98	48·5	12·6-102	21·8	Davies, 1975**

* Dry weight basis.
** Converted to 18·6% moisture for comparison with other data.

Materials and Methods

The method of Ough (1969) was selected because of its sensitivity, simplicity, and specificity.

* Research Fellow, Honey Industry Council, Inc.
** Agricultural Research Service, U.S. Department of Agriculture.

The principal interfering compounds are lysine, tryptophan and glutamine, with responses about 5%, 2% and 1·4% that of proline. Since the first two compounds comprise only about 0·8 and 3·2% of the free amino acids, respectively (Davies, 1975), this error is about 0·2% of the proline value, and may be disregarded. The error from glutamine is less than that from lysine, although honeys may contain more glutamine than lysine.

Method

Weigh 2·500 g honey, transfer it to a 50-ml volumetric flask and make to volume with water. Pipette 0·50 ml into each of three reaction tubes (20 × 150 mm borosilicate screw-cap culture tubes, caps with teflon lining), add 0·25 ml concentrated formic acid and 1·00 ml 3% ninhydrin in peroxide-free methyl cellosolve. Cap tightly, mix well, and place in a boiling-water bath for 15 min. Cool in a 22°C water bath for 5 min, add 5·00 ml of 1:1 (v/v) aqueous isopropanol to each. Mix well and measure absorbance at 520 nm, against a blank made as above but with water instead of honey solution. Read all tubes within 35 min of cooling. Correct for colour contribution of honey by determining absorbance of a mixture of 0·50 ml honey solution, 1·25 ml water, and 5·00 ml aqueous isopropanol. Subtract the value from the average of those found for the sample before calculating, using a calibration curve obtained with solutions of pure dry proline substituted for honey solutions.

Honey samples

Samples produced in 1956-1957. Portions of samples collected from producers for the study of honey composition reported earlier (White et al., 1962) had been kept in storage at −15 to −18°C for the intervening 20 years.

Samples produced in 1974-1975. These were collected for other work from producers who provided information on floral source, region of production, and heating and storage history. They were certified by the producers as genuine honeys. Samples were subdivided and portions were stored at approximately −18°C and 4°C. The latter were used for this analysis.

Feeding experiments

Four small colonies of bees on washed combs were fed with sucrose and high-fructose corn syrup of various solids contents (Table 4). Each was in a "mini-hive" (Waller, 1977) and contained about 225 g of bees with a caged queen. The hives were in separate flight cages 3·7 × 7·3 × 1·8 m. There was no access to pollen, and colonies were fed with the indicated syrup for 15 days. The stored material was removed; all was capped except that from feeding HFCS containing 30% solids.

Results and Discussion

Recovery of added proline

Five ml of a solution containing 42·3 μg proline/ml were added to 5-ml and 10-ml portions of a honey solution found by analysis to contain 30·8 μg proline/ml. The mixtures were found to contain 36·1 and 33·7 μg/ml proline, corresponding to recoveries of 98·9% and 97·3%.

The same sample of honey was analysed each day, as a control on the procedure. For 20 days the standard deviation of proline content was 0·28 mg/100 g.

The results of the analysis of the honey samples are summarized in Table 2, and the distribution of values for the 1974-75 samples is shown in Fig. 1. Examination of the data

by the F and t tests shows that the differences between the means and the standard deviations for the two groups are significant at the 0·1% probability level, so they are not combined in Fig. 1. The reason for the difference is not clear; storage or production factors could be responsible. The data on the 1956-57 samples provided additional information on samples whose composition was extensively recorded in an earlier survey (White et al., 1962).

TABLE 2. Proline content of U.S. honeys (mg/100 g), with standard deviation (SD) and coefficient variation (CV).

Crop year	*No. samples*	*Mean*	*Range*	*SD*	*CV*
1956-57	258	54·1	16·9-148·3	21·9	40·5%
1974-75	482	48·3	14·8-139	18·6	38·5%

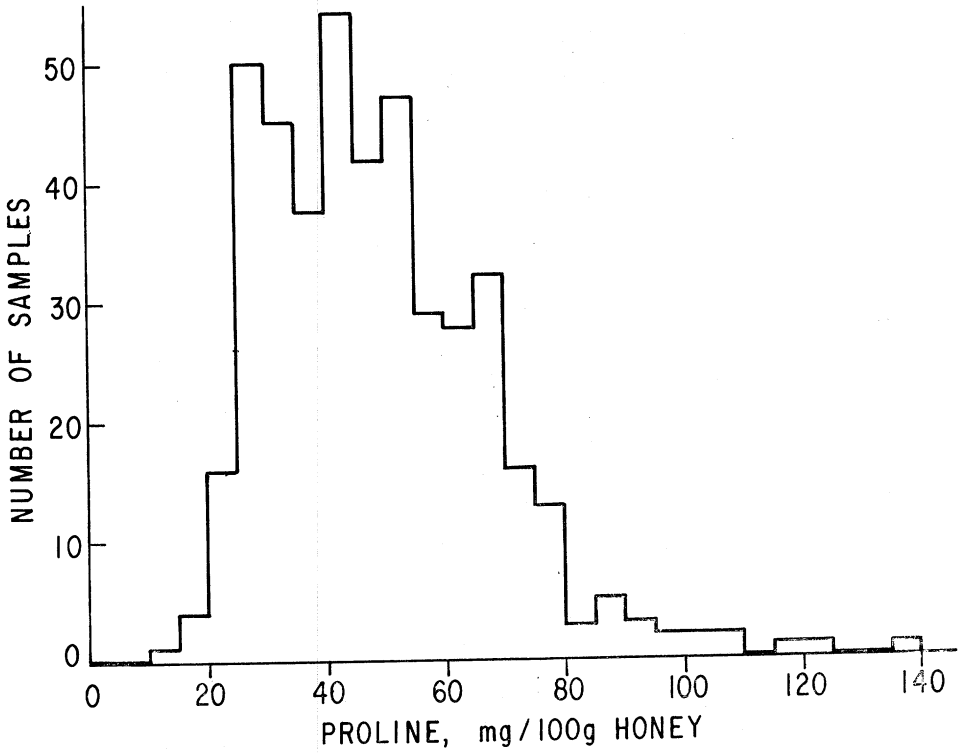

FIG. 1. Distribution of proline content of 482 samples of United States honeys (1974-75).

Effect of storage on proline content

Samples of three honeys which were subjected to storage at 37°C for an earlier study (White et al., 1964) had been stored at *c.* −18°C since that time. The controls, which had never been out of the freezer, and a set that had been stored at 37°C for 182 days, were analysed for proline. The average loss of proline in the samples stored at 37°C was 18·6% (Table 3).

TABLE 3. Effect of warm storage on the proline content of honey (mg/100 g). Samples were stored for 128 days at 37°C.

Sample no.	*Control*	*Stored*	*Loss*
1	41·9	36·0	14·1%
2	44·2	35·5	19·7%
3	47·7	37·2	22·0%

* See White et al., 1964.

Wootton et al. (1976) reported on the effect of storage for 44 days at 50°C on the free amino acids of six Australian honeys. Results varied widely, ranging from a 10% increase to an 85% loss of proline, but such storage conditions so far exceed any conceivable regimen for honey that they are not useful for control purposes. Assuming a logarithmic loss rate, the proline loss found in our work would have been reached in 6 days at 50°C; the range of values calculated by Wooton et al. for the four samples with the largest losses (12-22%) at that point compares well with the data in our Table 3. Bergner and Hahn (1972) reported a 15% loss of proline during storage of a honey for 42 days at 45°C. Both groups of workers ascribed losses to reaction with reducing sugars.

It is reasonable to conclude that storage normally encountered in honey trading will not cause significant loss in proline content. Abusive storage that would reduce proline significantly should greatly increase hydroxymethylfurfural content, to above 30 mg/100 g.

Minimum proline content

No samples were found to contain less proline than 14·8 mg/100 g honey (Table 2). The stores from colonies in another (feeding) experiment were analysed for proline. It was expected that these stores, from food with a higher content of solids than nectar, would exhibit the minimum level of proline: little manipulation was required to ripen them, all the more so because the colonies were kept in an area of low atmospheric humidity.

Bergner and Hahn (1972) fed a confined pollen-free colony with a "1:2" sugar solution and determined amino acids in samples of stores taken periodically for 55 days. Proline content, 29·9 mg/100 g by the third day, increased to 50·6 at 13 days and then declined; by 24 days it was less than 10. Stores in a free-flying colony fed the same solution, sampled after 4 and 30 days, contained 66·7 and 14·9 mg proline per 100 g respectively. The contents of the honey sac of captive bees fed 80% sucrose contained 26·4 mg/100 g; another determination with the same bees gave 17·3 mg/100 g.

It is evident from the data of Bergner and Hahn that, in long-confined colonies, availability of pollen (protein) strongly influences the proline content of their stored food. Under the conditions of our test, the proline content of the stores increased significantly as the solids content of the feed decreased (Table 4).

TABLE 4. Proline content (mg/100 g) in stores of colonies fed sugar solutions.

Feed material	*% Solids in feed*	*% Solids in stores*	*Proline*
High-fructose corn syrup	71	83·5	14·0
	50	82·6	15·1 z*
	30	82·4	16·6
Sucrose	50	80·5	15·4 z

* Data were processed by analyses of variance and Duncan's Multiple Range Test. Values not followed by a letter in common differ significantly at the 1% probability level.

The wide range of values found for the proline content of honey, and the minimum value 15 mg/100 g, appear to result from the interaction of a number of variables. The width of the range reflects the wide variety of nectar and of environmental conditions encountered in the United States.

Acknowledgements

We are indebted to the Honey Industry Council of America, Inc., for partial support of this work, and to Dr. Gordon Waller, USDA Bee Research Laboratory, Tucson, Arizona, for his co-operation in feeding sucrose and high-fructose corn syrup to caged colonies.

References

BERGNER, K.; HAHN, H. (1972) Zum Vorkommen und zur Herkunft der freien Aminosäuren in Honig. *Apidologie* 3:5-34

CURTI, R.; RIGANTI, V. (1966) Ricerche sugli aminoacidi del miele. *Rass. chim.* 18(6): 278-282

DAVIES, A. M. C. (1975) Amino acid analyses of honeys from eleven countries. *J. apic. Res.* 14(1): 29-39

KOMAMINE, A. (1960) Amino acids in honey. *Acta chem. fenn.* B33: 185-187

MAEDA, S.; MUKAI, A.; KOSUGI, N.; OKADA, Y. (1962) The flavor components of honey. *J. Fd. Sci. Technol.* 9(7): 270-274

MICHELOTTI, P.; MARGHERI, G. (1969) Studio sul contenuto in aminoacidi del miele. *Scienza Aliment.* 15(7): 179-180

OUGH, C. S. (1969) Rapid determination of proline in grapes and wines. *J. Food Sci.* 34: 228-430

PETROV, V. (1974) Quantitative determination of amino acids in some Australian honeys. *J. apic. Res.* 13(1): 61-66

WHITE, J. W., JR.; KUSHNIR, I.; SUBERS, M. H. (1964) Effect of storage and processing temperatures on honey quality. *Fd. Technol.* 18(4): 153-156

WHITE, J. W., JR.; RIETHOF, M. L.; SUBERS, M. H.; KUSHNIR, I. (1962) Composition of American honeys. *Tech. Bull. U.S. Dep. Agric.* No. 1261

WOOTTON, M.; EDWARDS, R. A.; FARAJI-HAREMI, R. (1976) Effect of accelerated storage conditions on the chemical composition and properties of Australian honeys. *J. apic. Res.* 15(1): 29-34